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DETECTION AND STUDY OF MICROORGANISMS IN THE UPPER ATMOSPHERE

UNPUBLISHED PRELIMINARY DATA

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2

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ABSTRACT

13980 over

Since the project report of August 20, 1962, research progress has been made in the following areas:

1. Refinement and modification of electrostatic precipitator configuration.

Due to difficulties in plating a satisfactory electrical conductor on the outer electrode (the precipitator tube) it was decided to investigate metal tubes. The use of metal tubes was found to greatly simplify electrical energization and also simplified the mechanical design and durability of the flight sampler. The use of non-transparent tubes also permitted a more flexible technique for bacteriological analysis of potential samples.

2. Generation of Test Aerosols.

Some difficulty was encountered in generating dry aerosols due to the static charge acquired by the particles during dispersal. This static charge caused the particles to cling to the walls of the mixing chamber. A large chamber in which it is hoped to minimize the static charge problem is now under construction. It is designed to permit generation of both wet and dry aerosols.

3. Study of Electrical Characteristics of the Electrostatic Precipitator under Low Air Density Conditions.

Tests have been conducted to find the voltage current relations for a model precipitator operated under low air density conditions. To date, these experiments have given encouraging results in that necessary power levels are small and operational stability is satisfactory.

4. Design of Flight Sampler. 13980

Coincident with the laboratory experiments work has continued on 15 2" ID by 24" long aluminum precipitator tubes in a cylindrical casing. Experiments are now underway to develop a reliable sealing system. The selection of commercially available components is also being carried out.

5. Development of Bacteriological Techniques.

Considerable work has been done in the development of techniques for the generation of aerosols, and comparative methods for evaluation of results of the laboratory precipitator experiment. Tests have been made with an Andersen Sampler to determine its usefulness as a comparative control sampler. The analysis procedure to be used on the flight samples has been developed and will be tested upon completion of the aerosol chamber.

I. REFINEMENT AND MODIFICATION OF ELECTROSTATIC PRECIPITATOR CONFIGURATION

The precipitator tube as originally designed utilized a glass collecting tube. It was proposed to culture and count the colonies in this tube prior to opening it for the removal and identification of the microorganisms. Several types of electrically conducting coatings that were transparent were investigated, tin oxide being the most promising. To achieve a satisfactory coating on the glass using tin oxide, it is necessary to heat the tube to 500°C. A small resistance heater was built for this purpose. Tubes were then heated and the coating vapor generated by heating a flask of $SnCl_4 \cdot 5H_2O$ and phenylhydrazine hydrochloride was passed into the tubes. Fifteen to twenty tubes were coated using this method. However, the quality of the coating was very inconsistent, ranging from fair to very

poor. It was found that the temperature of the glass was much more critical than had been assumed and that the heater was unsatisfactory to maintain the necessary temperature over the entire tube.

Simultaneously, doubts were raised as to the suitability of glass to withstand impact loads that might be encountered upon landing a flight sampler. It was also pointed out that the technique of culturing organisms in the tube had certain disadvantages with respect to achieving dilutions if a large number of organisms were collected. Notwithstanding these objections, an attempt was made to achieve a satisfactory coating by vacuum plating aluminum on the glass tubes. This method was also unsatisfactory due to an inability to control the thickness of the aluminum to achieve the necessary electrical properties as well as reasonable optical characteristics.

In view of the above difficulties, a brief study was made of the various simplifications and/or complications that would arise from the use of metal tubes, specifically aluminum. The evidence is that this change has bypassed the plating and durability problems of glass tubes, and in addition, allows a more flexible technique for bacteriological analysis (see part V).

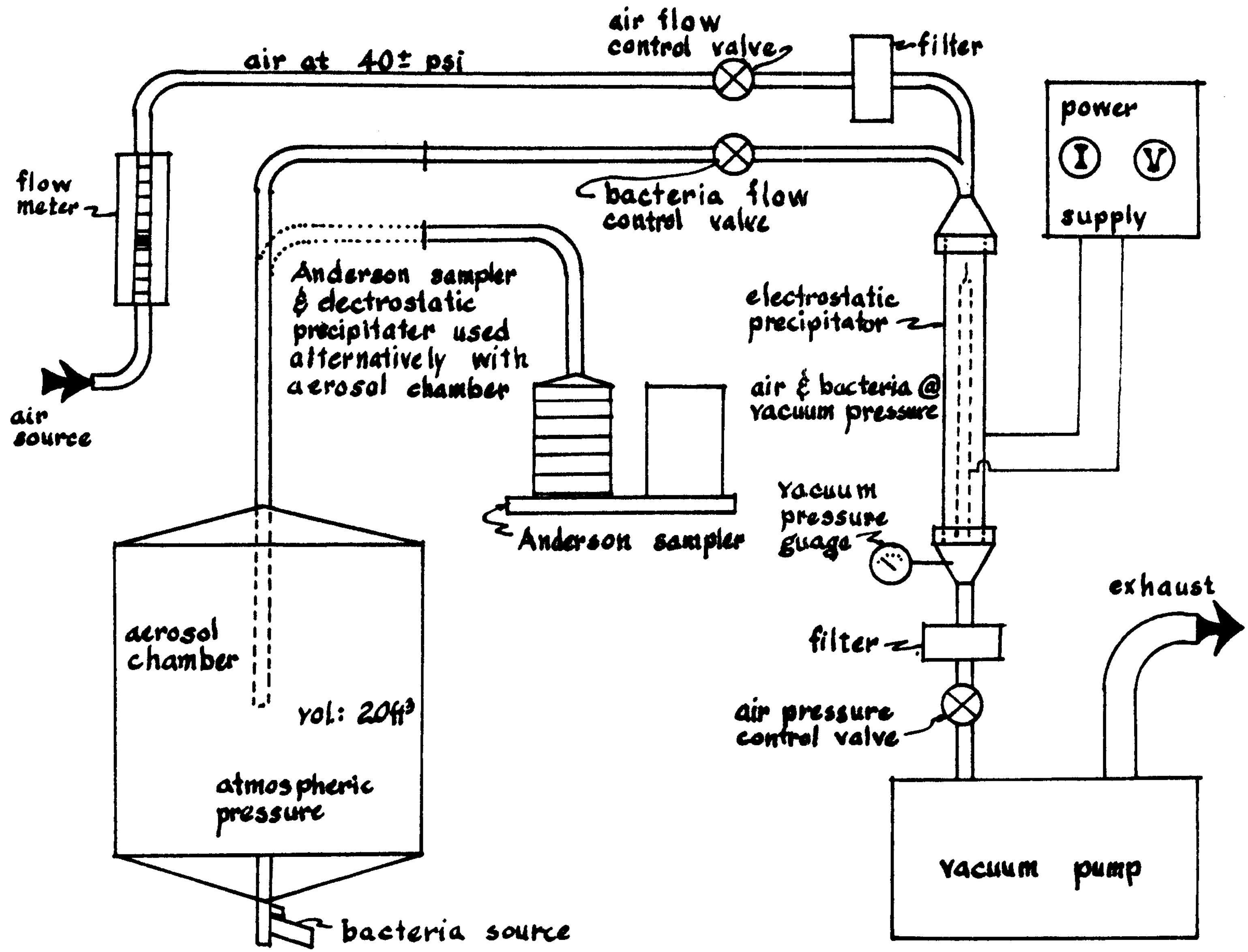
II. GENERATION OF TEST AEROSOLS

A literature survey was conducted to determine a suitable method for the generation of aerosols for injection into the experimental precipitator. It was found that for a reasonable retention time the aerosol cloud should be generated at atmospheric pressure and then injected into the low-pressure portion of the system. Furthermore, it was decided to concentrate on dried micro-organisms as being representative of those likely to be encountered at high altitudes.

A number of pilot experiments were conducted using a conventional osterizer to generate aerosols. A dry suspension of lyophilized Bacillus subtilis spores was introduced into the osterizer and agitated for varying lengths of time. Samples were then removed with a #16 long hypodermic needle and a sterile syringe. Analysis of the contents of the syringe showed that no organisms were contained in the sample air. However, when the inner surfaces of the blender were washed with sterile saline, subsequent plating revealed that 5×10^5 micro-organisms had been introduced. It was found that a static charge was being accumulated by the particles due to the action of the metal agitator blade of the blender. It was also found that the lyopylized particles absorbed water. Both these effects tended to cause the organisms to precipitate and stick on the walls of the blender. A further survey of the literature and consultation with the Naval Biological Laboratories, University of California, showed that both of these effects are general problems in the generation of dry aerosols, and that the best means of minimizing these effects is through the use of a large metal chamber with the proper humidity controls. Such a chamber has been designed and is now under construction.

The metal chamber will be suitable for the generation and retention of both wet and dry aerosols. The chamber volume is 20 cu.ft. Dry dusts will be injected by firing a gelatin capsule containing the dust from a CO_2 pistol into the chamber. When the capsule enters the chamber it crosses a razor blade and the dust is explosively released. In preliminary runs this method has been promising. Wet suspensions will be injected using a Vaponephrin nebulizer which has also been tested and found suitable for these studies.

Figure #1 Schematic of Elecrostatic Precipitator Test Apparatus



In the collection efficiency test runs the aerosols produced in the chamber will be measured alternately with the precipitator and the Andersen Sampler (Ref. 1). The Andersen Sampler will thus serve as a control device with well-established characteristics when operated at atmospheric pressure, whereas the precipitator will be operated at the simulated altitude pressure (Fig. 1). Test runs will be made using known lyophilized bacteria as well as pulverized soils. The use of soil aerosols will check the relative similarity in the types of micro-organisms recovered by the two methods and serve as a testing ground for the techniques to be used for the analysis of the flight samples.

To perfect the operational manipulation of the Andersen Sampler for our purposes, Vaponephrin Nebulizer aerosols of B. subtilis spores were used. In many of the trials, the viable counts on the sixth stage of the sampler were so high, that it could be implied that not all the organisms were being collected. Thus, for the actual running of the efficiency test, a seventh stage in the system will be added as a further control. This stage will be a Type AA millipore filter, connected to a flow meter and a pump of greater capacity to insure an optimum flow rate of 28.3 liters/min.

III. PRECIPITATOR ELECTRICAL CHARACTERISTICS

The primary goal in this series of tests was to determine the power levels and voltage current characteristics of an electrostatic precipitator of the concentric electrode type with a view towards determining the necessary design data for the construction of a flight sampler. To date no attempt has been made to develop a mathematical model to fit the experimental data nor has any attempt been made to explain the experimental

results in terms of the underlying physics of gaseous discharge. However, the testing program that has been used was selected so that the data would be in a form amenable to statistical analysis and ultimate reduction to analytical form.

For a precipitator operated at atmospheric pressure and with the physical dimensions of the model used a typical voltage level is 20,000 volts D.C. At this voltage and pressure a current of 250 micro amperes was recorded. Hence, the range of current selected for investigation was 0 to 500 micro-amperes. The range of flow rates was determined by the physical capability of the vacuum system which was designed to give collection efficiencies of 20 to 100% based on the equations developed by White (Ref. 2) for atmospheric pressure operation. The voltage was supplied by an unregulated D.C. source. Flow was measured by a Fischer & Porter Rotameter[(1)] operated at 50 psia and the pressure measured by a mercury manometer or a Hastings vacuum gauge[(2)].

A number of test runs were conducted holding two variables constant and investigating the relation between the remaining variables. The most interesting results of these tests are shown in Figs. 2 - 5. These data indicate that the voltage-air pressure relationship is nearly linear for constant current and constant gas flow and that the effect of gas flow rate on voltage is very small in the range investigated. However, it should be noted that the slope of the approximate straight line voltage pressure relationship changes appreciably between Figs. 2 and 3. It is not clear

[(1)] Flowrater meter, Model 10A2700, Fischer & Porter Co., Warminster, Pa.

[(2)] Vacuum gauge, Model SP-1, Hastings Raydist, Inc., Hampton, Va.

whether the flow change from 50% to 15% is responsible for this effect or whether a different discharge phenomena occurred due to the decrease in pressure.

The parametric approach described above necessitated a rather involved testing program which did not lend itself directly to statistical analysis. Therefore, it was decided to design a test program using the method of orthogonal squares (Ref. 4) which is based on statistical methods and can be used to reduce the data to analytical form. This method finds its greatest utility in situations where the independent variables are truly independent, i.e., where there are no coupling effects.

The atmospheric range from 10 to 4 inches of mercury absolute has been investigated by the method of orthogonal squares. The pressure range studied corresponds to an altitude range of about 27,000 to 47,000 feet. In these tests current was treated as the dependent variable with voltage, pressure and flow rate the independent variables.

The initial set of orthogonal squares was selected on the assumption that coupling effects among the variables would be small. Five by five orthogonal squares were used in order to limit the current to 500 μ amps and to conform with equipment limitations. The results of two runs are presented in Figs. 6 - 11.

Although the analysis of the data presented in Figs. 6 - 11 has not been completed, several effects are quite obvious. The most evident finding is that the effect of flow rate on current is larger than had been expected. This finding is opposed to that obtained by the conventional parametric variation technique shown in Fig. 4. Hence, it is conceivable

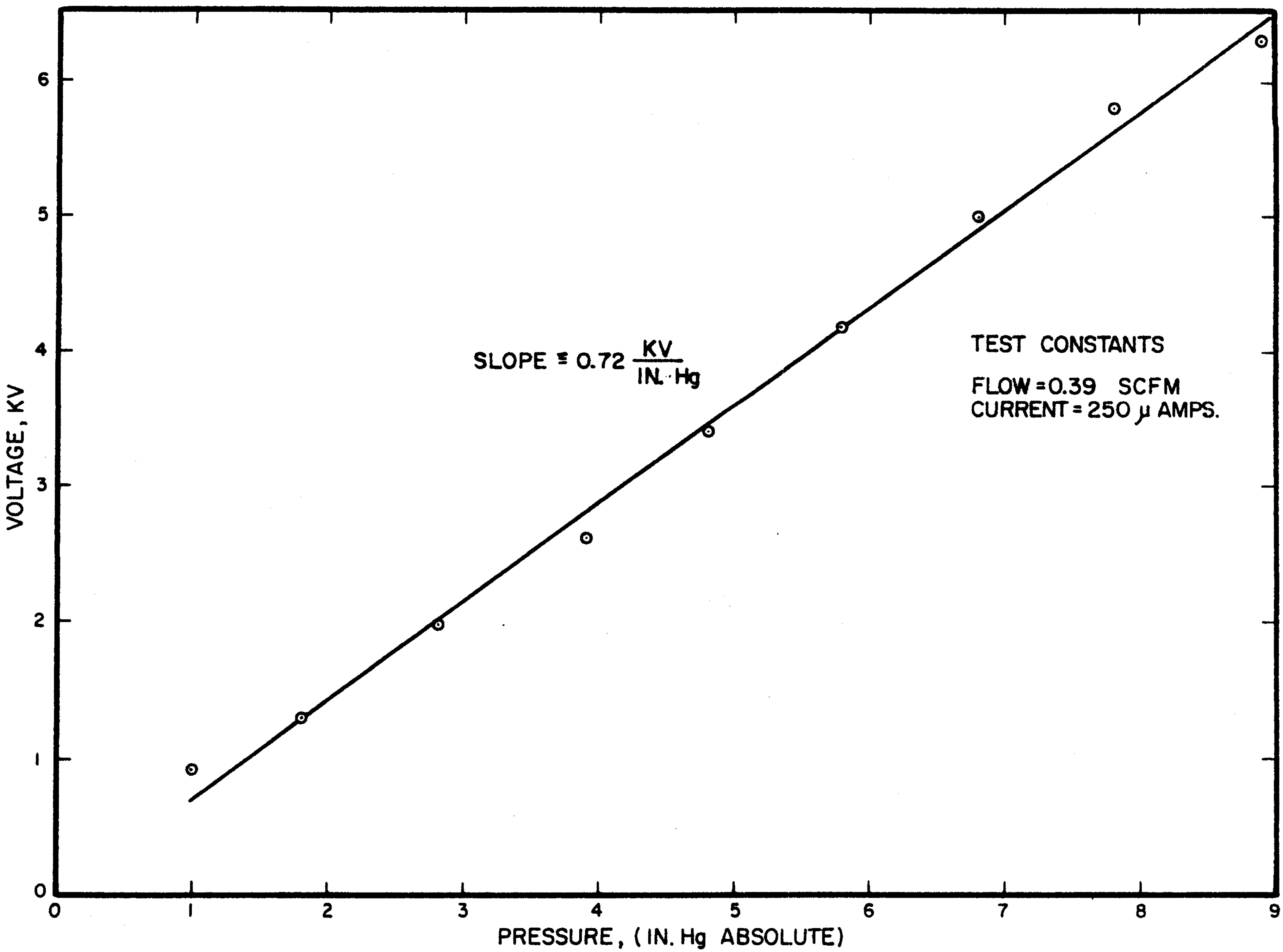
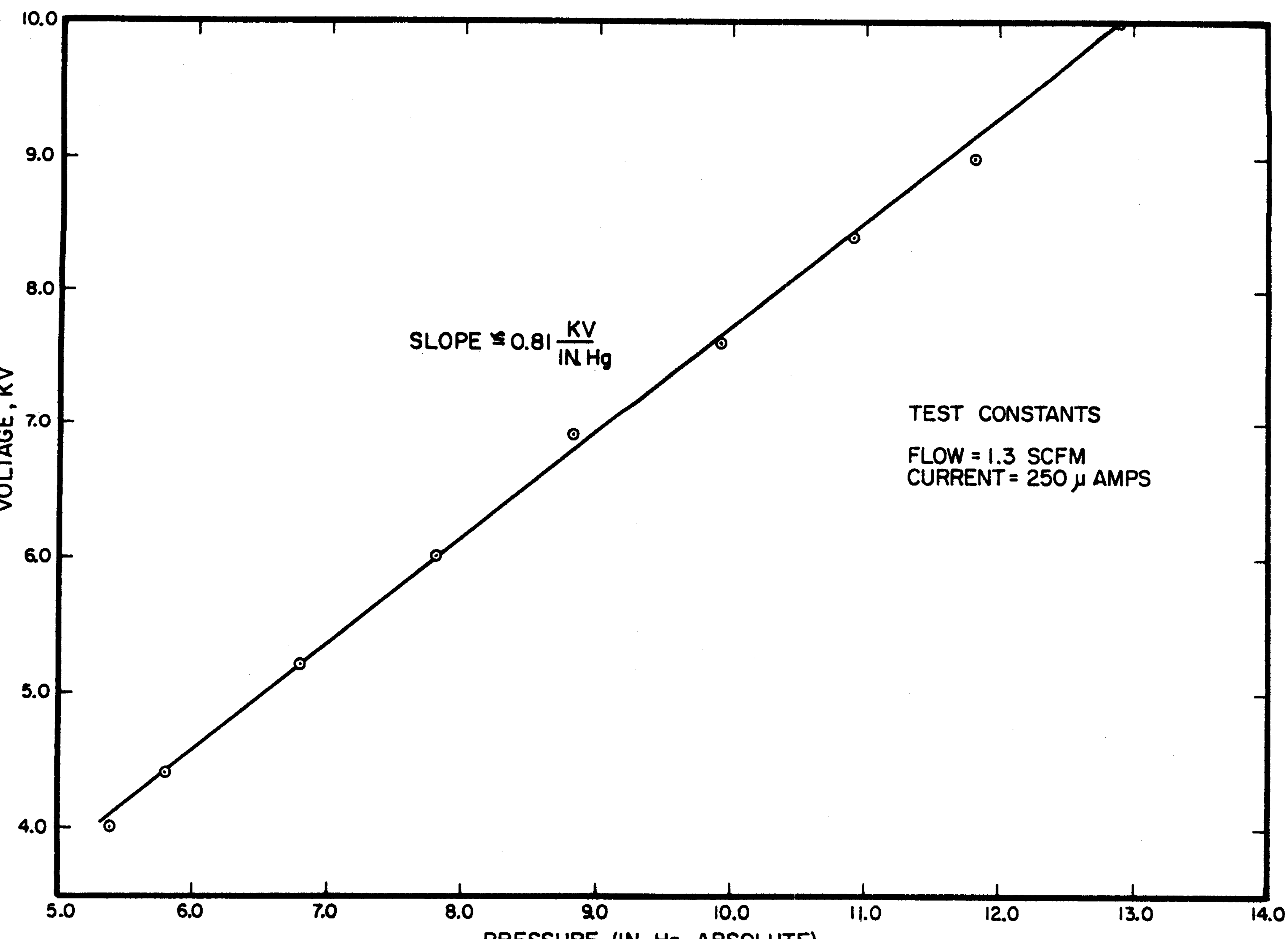

SLOPE ≅ 0.72 KV/IN. Hg
TEST CONSTANTS
FLOW = 0.39 SCFM
CURRENT = 250 μ AMPS.
VOLTAGE, KV
PRESSURE, (IN. Hg ABSOLUTE)
0
1
2
3
4
5
6
7
8
9

FIGURE 2



VOLTAGE, KV
10.0
9.0
8.0
7.0
6.0
5.0
4.0
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
PRESSURE, (IN. Hg ABSOLUTE)
SLOPE ≅ 0.81 KV/IN. Hg
TEST CONSTANTS
FLOW = 1.3 SCFM
CURRENT = 250 μ AMPS

FIGURE 3

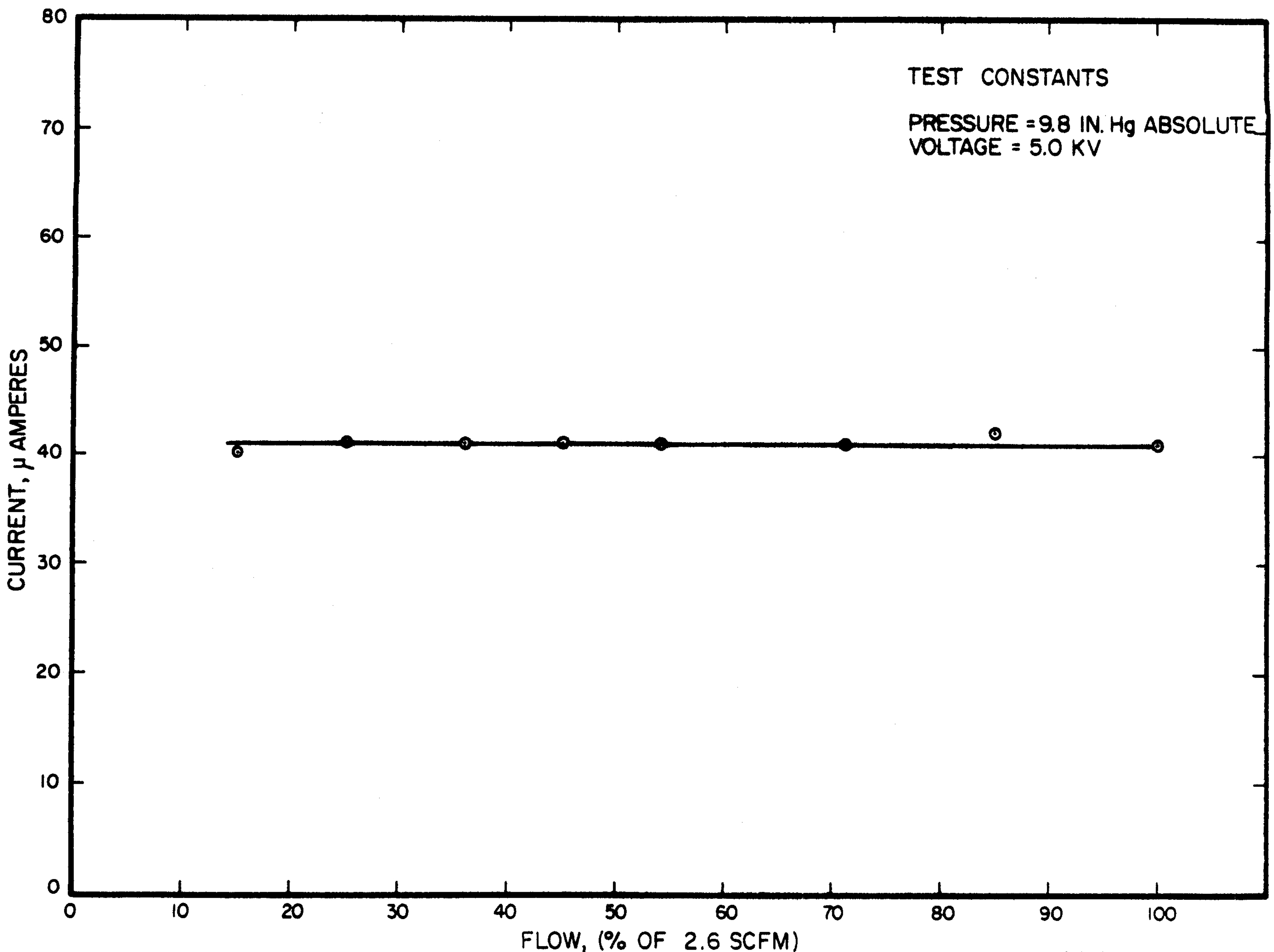

TEST CONSTANTS
PRESSURE = 9.8 IN. Hg ABSOLUTE
VOLTAGE = 5.0 KV
CURRENT, μ AMPERES
0
10
20
30
40
50
60
70
80
0
10
20
30
40
50
60
70
80
90
100
FLOW, (% OF 2.6 SCFM)

FIGURE 4

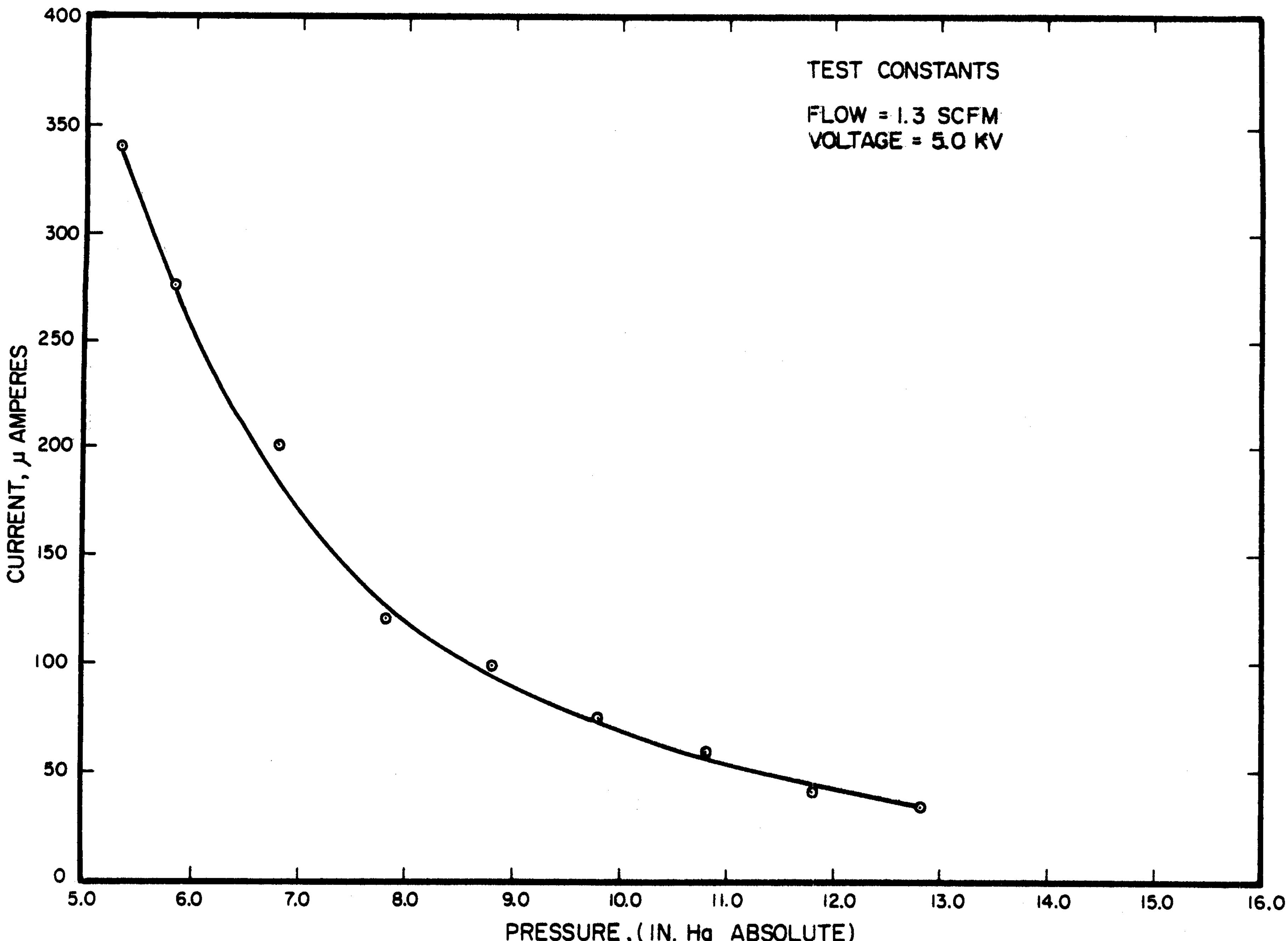

TEST CONSTANTS
FLOW = 1.3 SCFM
VOLTAGE = 5.0 KV
CURRENT, μ AMPERES
0
50
100
150
200
250
300
350
400
5.0
6.0
7.0
8.0
9.0
10.0
11.0
12.0
13.0
14.0
15.0
16.0
PRESSURE, (IN. Hg ABSOLUTE)

FIGURE 5

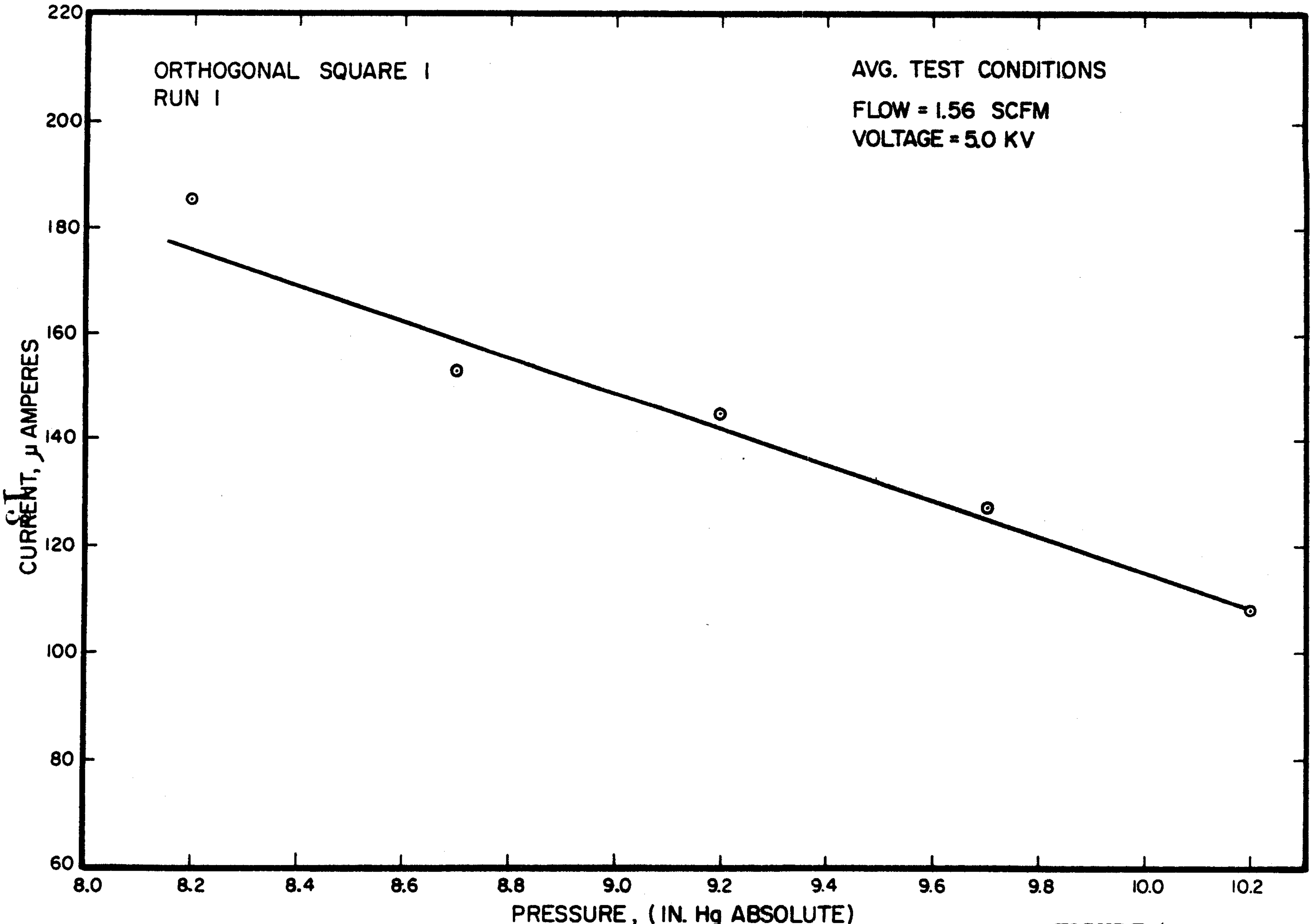

ORTHOGONAL SQUARE I
RUN I
AVG. TEST CONDITIONS
FLOW = 1.56 SCFM
VOLTAGE = 5.0 KV
CURRENT, μ AMPERES
220
200
180
160
140
120
100
80
60
8.0
8.2
8.4
8.6
8.8
9.0
9.2
9.4
9.6
9.8
10.0
10.2
PRESSURE, (IN. Hg ABSOLUTE)

FIGURE 6

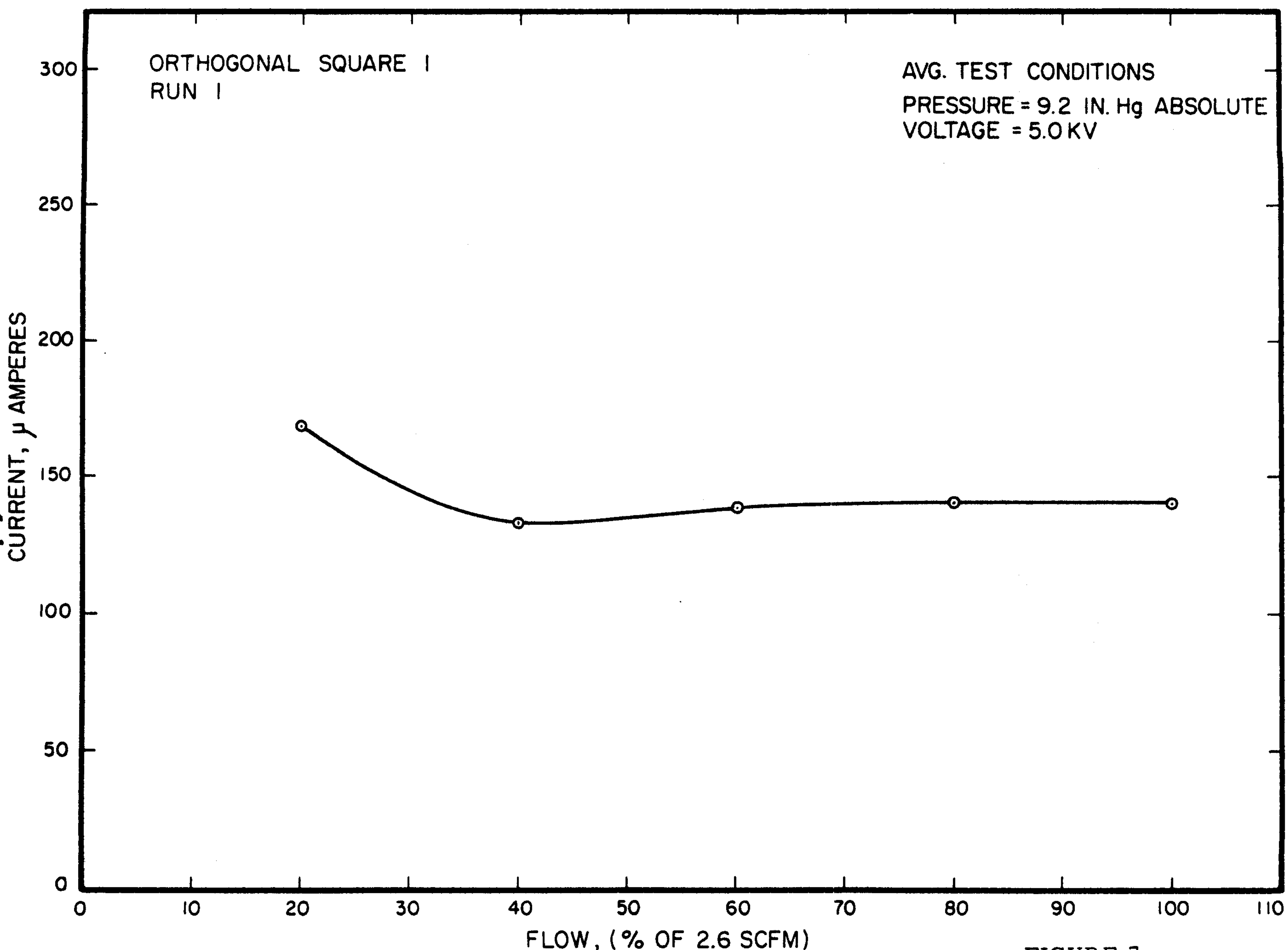
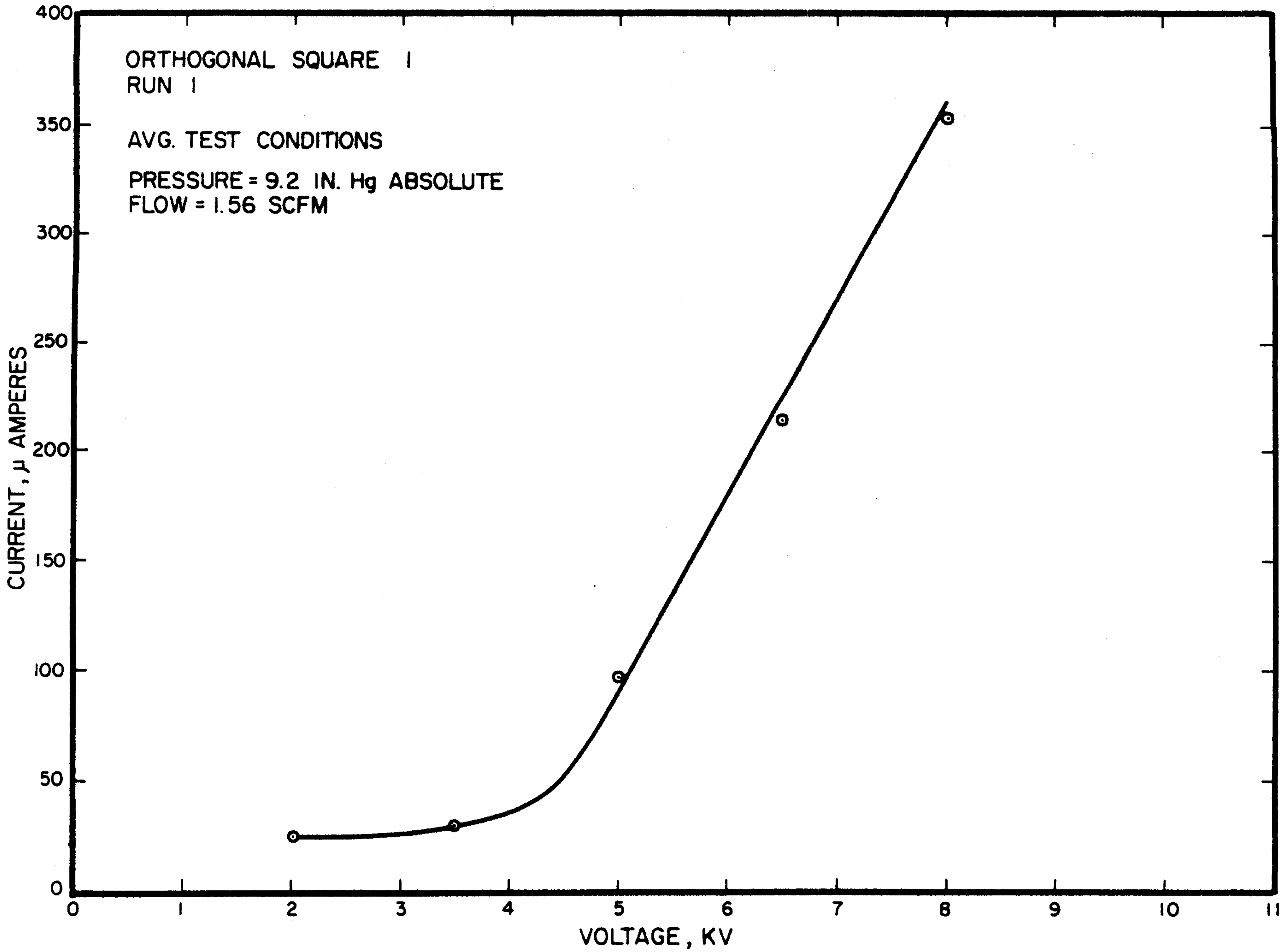

ORTHOGONAL SQUARE I
RUN I
AVG. TEST CONDITIONS
PRESSURE = 9.2 IN. Hg ABSOLUTE
VOLTAGE = 5.0 KV
CURRENT, μ AMPERES
0
50
100
150
200
250
300
0
10
20
30
40
50
60
70
80
90
100
110
FLOW, (% OF 2.6 SCFM)

FIGURE 7



ORTHOGONAL SQUARE I
RUN I
AVG. TEST CONDITIONS
PRESSURE = 9.2 IN. Hg ABSOLUTE
FLOW = 1.56 SCFM
CURRENT, μ AMPERES
VOLTAGE, KV
0
50
100
150
200
250
300
350
400
0
1
2
3
4
5
6
7
8
9
10
11

FIGURE 8

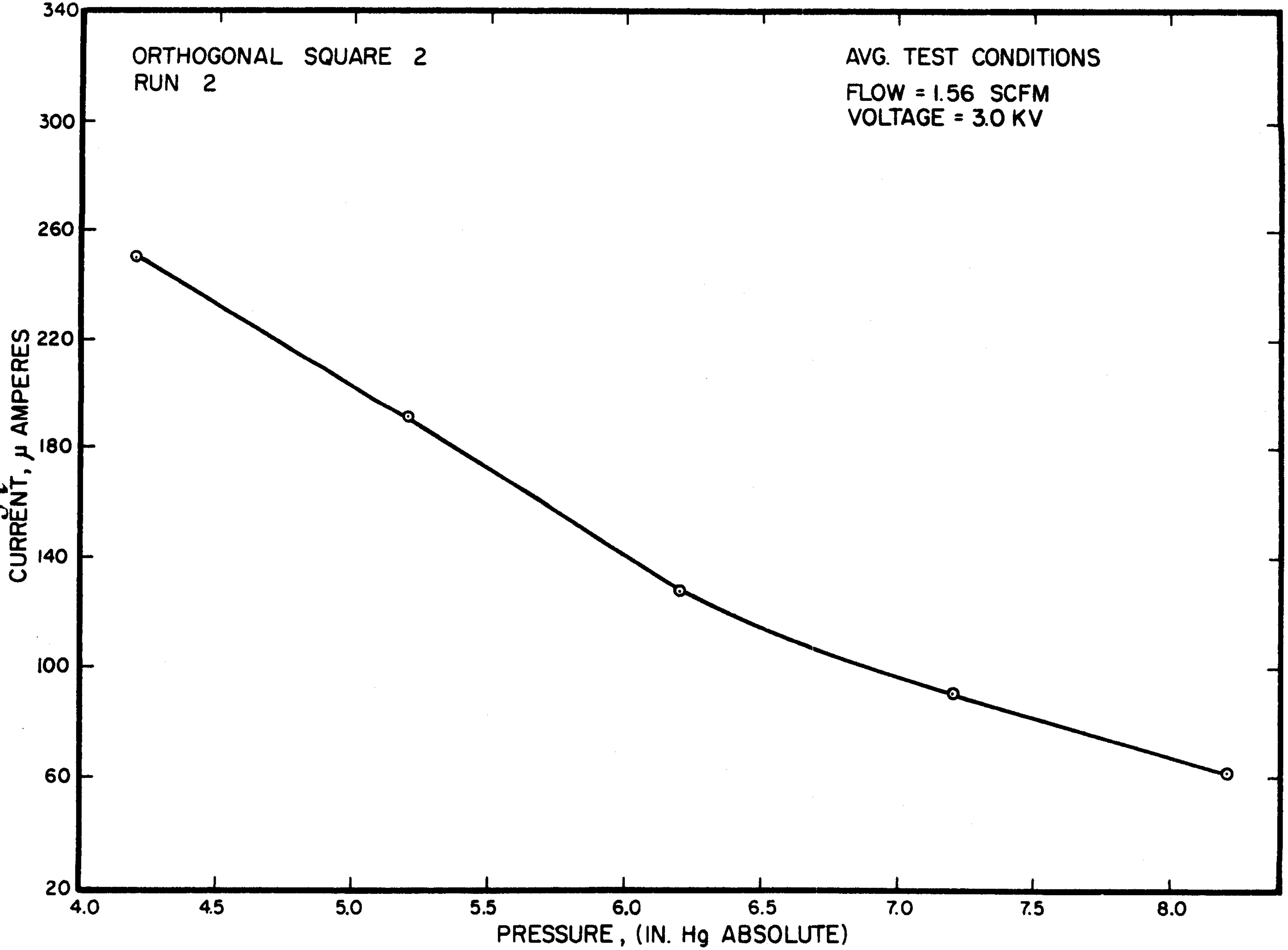

ORTHOGONAL SQUARE 2
RUN 2
AVG. TEST CONDITIONS
FLOW = 1.56 SCFM
VOLTAGE = 3.0 KV
CURRENT, μ AMPERES
340
300
260
220
180
140
100
60
20
4.0
4.5
5.0
5.5
6.0
6.5
7.0
7.5
8.0
PRESSURE, (IN. Hg ABSOLUTE)

FIGURE 9

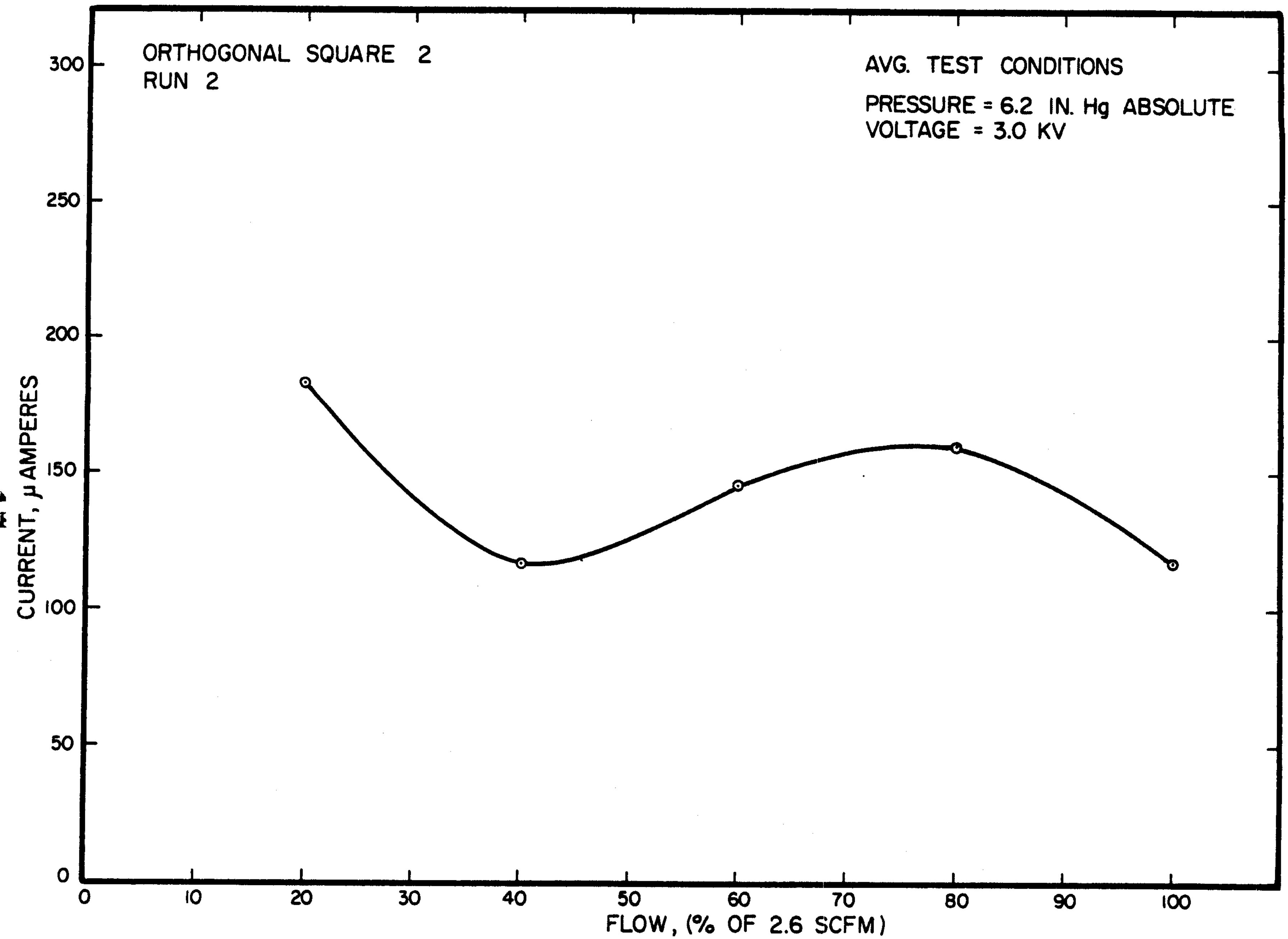

ORTHOGONAL SQUARE 2
RUN 2
AVG. TEST CONDITIONS
PRESSURE = 6.2 IN. Hg ABSOLUTE
VOLTAGE = 3.0 KV
CURRENT, μ AMPERES
0
50
100
150
200
250
300
0
10
20
30
40
50
60
70
80
90
100
FLOW, (% OF 2.6 SCFM)

FIGURE 10

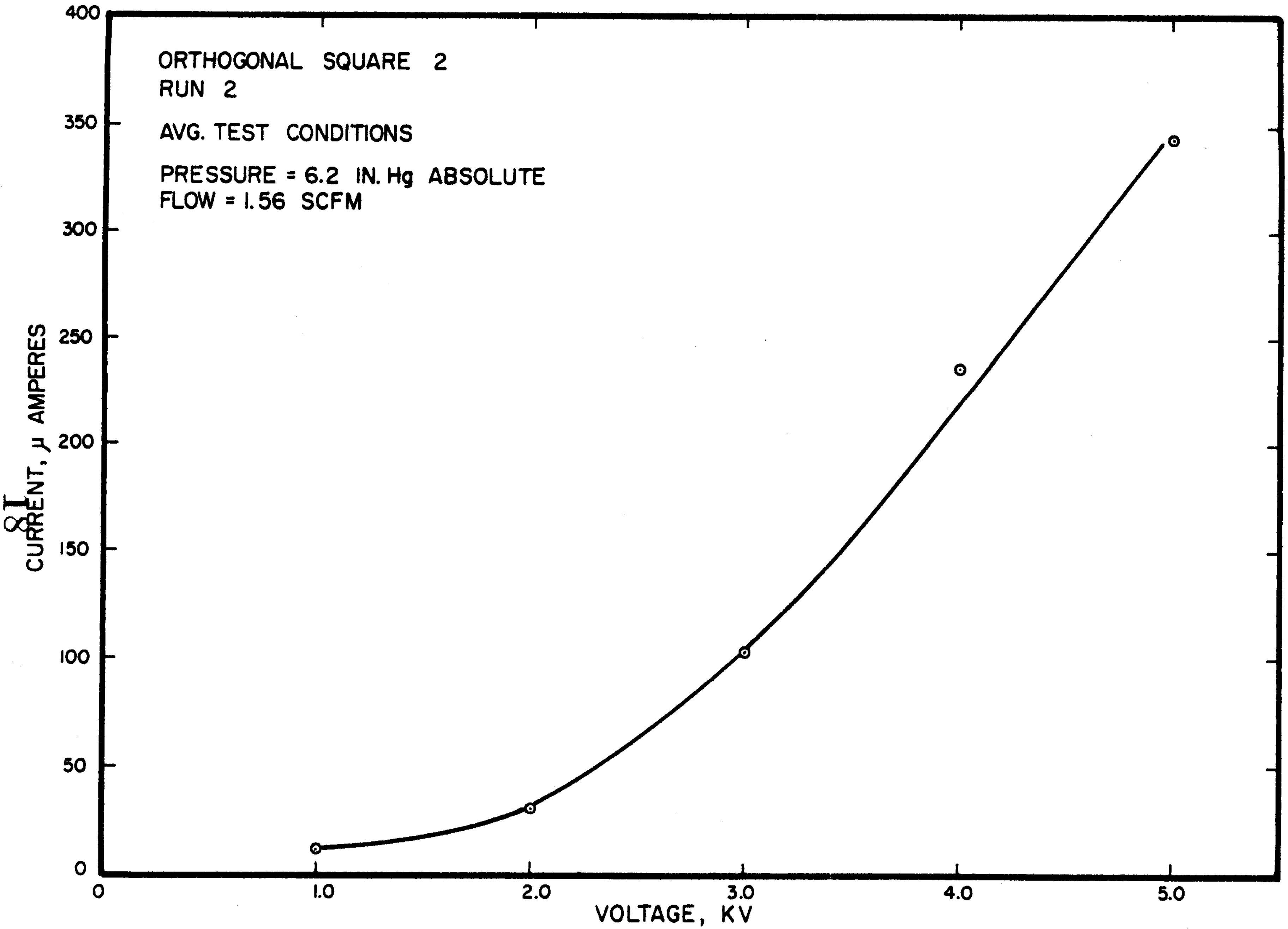

ORTHOGONAL SQUARE 2
RUN 2
AVG. TEST CONDITIONS
PRESSURE = 6.2 IN. Hg ABSOLUTE
FLOW = 1.56 SCFM
CURRENT, μ AMPERES
0
50
100
150
200
250
300
350
400
0
1.0
2.0
3.0
4.0
5.0
VOLTAGE, KV

FIGURE 11

that the variation shown by the orthogonal square method is not an actual physical effect, but is introduced by the method itself, perhaps due to interaction of variables. Further experimentation is in progress to resolve the different results yielded by the two methods.

The voltage versus current curves indicate that the power level per tube in the altitude ranges above 45,000 feet will be less than 1 watt for a 1" discharge wire. Furthermore, the discharge appears to be quite stable at 250μ amps throughout the entire range of pressures tested to date. It has been found that the "stability limit" of the discharge seems to largely a function of current and occurs at approximately 450 to 500μ amps. Stability to breakdown will be investigated in detail subsequent to the completion of the orthogonal square tests.

Inasmuch as the corona discharge of the precipitator produces ozone, a bacteriocidal gas, these experiments will be continued and include investigation of the degree of ozone generation in the precipitator and its effect on resting bacteria. Work is now underway to determine techniques for ozone measurement at low pressures. In these tests ozone concentration will be treated as the dependent variable, with pressure, current, and flow rate as the independent variables. In future experiments viable bacterial collection efficiency will be treated as a dependent variable with current (ozone), flow and pressure the independent variables.

IV. FLIGHT SAMPLER DESIGN

The design of a flight sampler is being considered. Ideas concerning the form of the sampler are accumulating in part from data obtained from previous investigators, and in part from the results of the electrostatic precipitator tests. Currently several tentative designs have been evaluated.

Design assumptions which have been established particularly with respect to balloon flight requirements are: testing sequence, pressure differentials, temperature variations, and the intensity of impact loading. The design assumptions are:

1. The testing altitude of maximum interest will vary from 44k to 130k ft. The sample will be collected while the balloon is in descent in order to insure the sampling of air uncontaminated by the balloon.

2. The pressure and temperature assumed are as given in the ARDC model atmosphere.

3. The precipitator tubes will be unsealed only during the sampling period of the flight and will be kept at the sampled pressure until bacteriological tests are initiated in the laboratory.

4. Impact loading calculations are based on a rate of descent of 1,000 fpm, which is equivalent to a free fall of five feet.

The sampler has been designed for single altitude samples or sequential samples at a series of altitudes. For the present, emphasis is being placed on a single altitude probe in order to take advantage of "piggyback flights" which may be available. Such flights would serve to prove the usefulness of the sampler and should also permit collection of bacteriological samples at a minimum cost.

Since reliability and simplicity are generally associated, the complexity of the apparatus and of its operation will be kept to a minimum.

In one version of a sampler fifteen 2" ID x 24" aluminum tubes are arranged evenly on the surface of a cylinder which has room for 30 tubes. The caps of the cylinders are attached to a circular plate at each end covering all fifteen of the tube ends. At the start of collection, the circular plates would extend as a result of the reduction in external air pressure, causing the tube caps to pull free of the tube ends. The circular plates would then rotate 12° by cam guided spring action so that each tube end would be over one of fifteen holes in the circular plate. The plates would then retract below the tube inlet, leaving a clean air stream approaching the tubes. At the end of the testing period, the same process would be repeated in reverse so that tubes would be resealed during descent. To avoid reverse rotation each tube would have the cap which had been on the tube next to it during ascent. The sealing requirements for tube caps are now under study, and tests are being planned now to establish which, of several, sealing mechanisms will be most effective and reliable. To do this a single tube is being constructed with capping and collection equipment.

It is expected, from the results of our laboratory tests on pressure, voltage and current that the precipitator power will not exceed several watts per tube. The total power requirements for the complete package described above will be determined as the laboratory precipitator experiments progress. The total power supply requirement may depend to a great extent on the air blower power requirements, which in turn depends entirely on the flow characteristics found from the results of our present tests. Thus a series of increasingly effective designs will be undertaken.

For the power and blower systems commercially available components will be used where possible. The mechanical assembly of the various models will, however, have to be constructed in the University shops.

Controls for the sampling system are currently visualized to include environmental sensors for the test commencement signal, electrical measuring devices, a motor driven relay system giving signals to the tube opening device, the electrical equipment, and the blower system. A camera will be used for recording all pertinent in-flight data. It is planned that controls, power pack and instrumentation will fit inside the cylinder formed by the ring of aluminum sampling tubes.

The advantage in the sampling device described lies in the fact that the tubes will be sealed before and after collection and will remain sealed until the organisms are cultured and sub-cultures are removed through a diaphragm under aseptic conditions. This will obviate the need to expose the contents of the tubes to questionable sterile techniques such as "white room" sterility. For ease of manipulation in the bacteriological laboratory, each sample tube will be dismountable from its support, and each cap, although fastened to the cover plate for flight operation, will be firmly attached to the sample tube by procedures carried out before bacteriological sampling begins. The caps are fitted with threaded ports leading to a penetrable diaphragm which will be punctured for the addition of sterile water and for the removal of the organisms for bacteriological testing.

V. MICROBIAL RECOVERY TECHNIQUES

A. Use of a solid media to enumerate and identify micro-organisms impinged on the electrostatic tube.

To test the recovery of bacteria in the electrostatic precipitator, preparation of the agar collector surface was by the method of Houwink and Rolvink (Ref. 3). Room air was used for sampling. After incubation at 30°C for 24 hours the collector tube showed abundant confluent growth of various aerobic bacteria. On further testing it was found that the agar coating would not withstand the pressures used at simulated altitudes.

A means of coating the tube for good impaction and then opening it aseptically and coating with agar after the test run was perfected. Silicone grease was applied in a thin coating to the inside of the tube. After the test run, with room air, the tube was removed and opened in Bacteriology Isolation/Lab Unit(1). Warm melted agar was added and coated with the usual roll-tube technique. The tube was then plugged with sterile No. 11 rubber stoppers and incubated. After 24 hours many colonies were seen. However, much confluent growth occurred.

While the technique of Houwink and Rolvink would undoubtedly pick up any micro-organism that had impinged on the tube by electrostatic force, it would not lead to accessibility to the colonies themselves, and thus make identification difficult. At atmospheric levels, where many micro-organisms might be encountered, this technique would lead to difficulties in the actual counting, since it is hard to prevent the colonies from converging. However, an excessive number of bacteria is not a likely problem

(1)Bacteriology Isolation/Lab Unit No. 32957 Kewaunce Mfg. Co., Adrian, Mich.

in samples culled from the upper atmosphere.

B. Use of a liquid media to enumerate and identify micro-organisms impinged on the electrostatic tube.

The inner surface of the tube is coated with silicon-grease. After the test run it is removed to the Isolator/Lab Unit, opened aseptically, and plugged with Sterile No. 11 one-hole stoppers, and plugged with cotton. A tryptose-phosphate diluent is added through one opening. The tube is thoroughly rinsed and the broth removed through the opposite side, using a stopcock sterile syringe technique. The washings are then plated on various media.

Trials were made using an aerosol of a 1:10 dilution of B. subtilis spores. The aerosol was blown directly into the silicone greased tube. This was allowed to dry for three hours. Sterile washings were then made and removed aseptically. One and five cc amounts were plated on nutrient agar. After 24 hours incubation good growth was seen. This technique was further tested as to recovery efficiency. B. subtilis spores (6×10^5) were introduced into the tube as before. Using the usual viable plate count technique, 4.5×10^5 were recovered. Loss was probably due to incomplete washing of the coated tube, since water tends to collect in droplets on the silicone grease.

Future techniques will utilize a Membrane Filter Bacteriological Analysis Monitor attached to one end of the Electrostatic Tube. This will be attached after the trial runs and removal of the tube from the Electrostatic Precipitation Apparatus in the Isolator/Lab Unit. A larger quantity of diluent will be employed in a series of washings. After each washing

a new filter will be used, until negative results are obtained. This should ensure adequate washing and the complete removal of all organisms introduced into the system.

Since the actual probe unit will contain fifteen tubes, it is planned to divide the tubes into three groups for more complete microbial analyses. Five of the tubes will be washed with thioglycollate broth and filtered through membrane filters which will be incubated on Brewer Anaerobic Agar, using the Brewer Anaerobic cover as described by Brewer (Science, 95:587: 1942). Five will be washed with tryptosephosphate broth, filtered through membrane filters and incubated on mold-yeast agar. The last group will be incubated on total count media for the enumeration and identification of aerobic bacteria.

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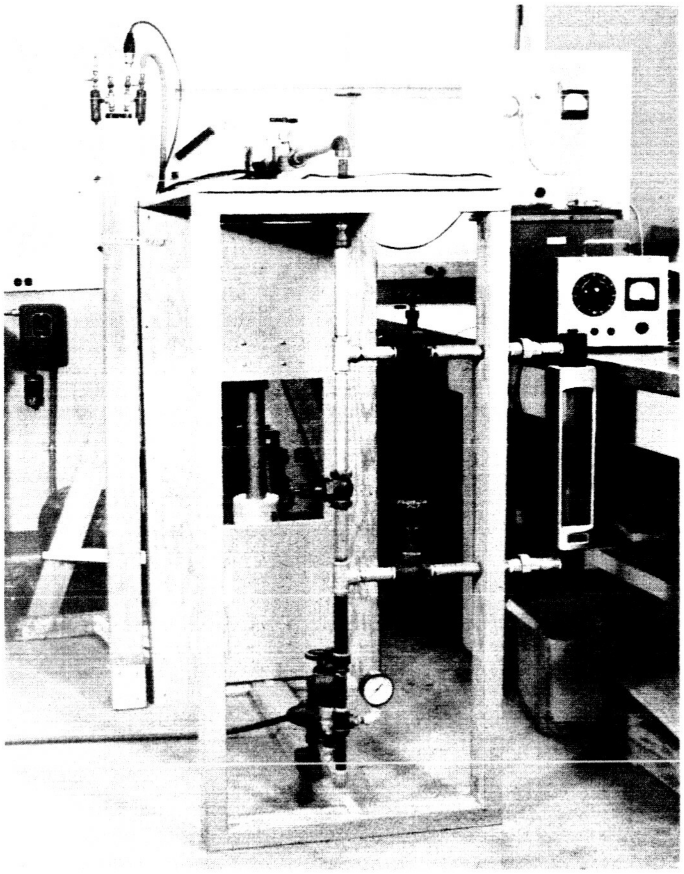
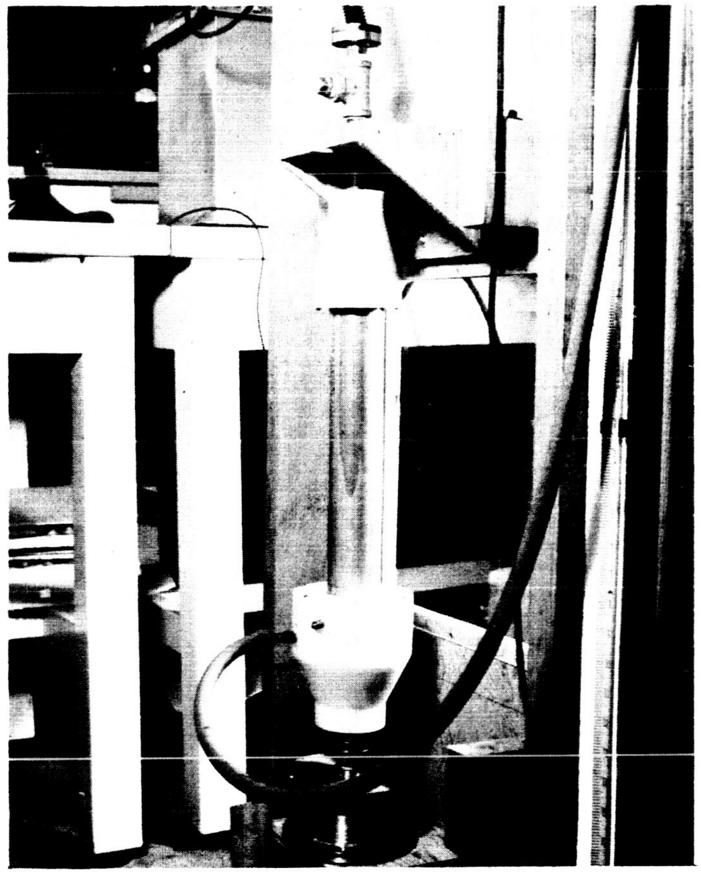
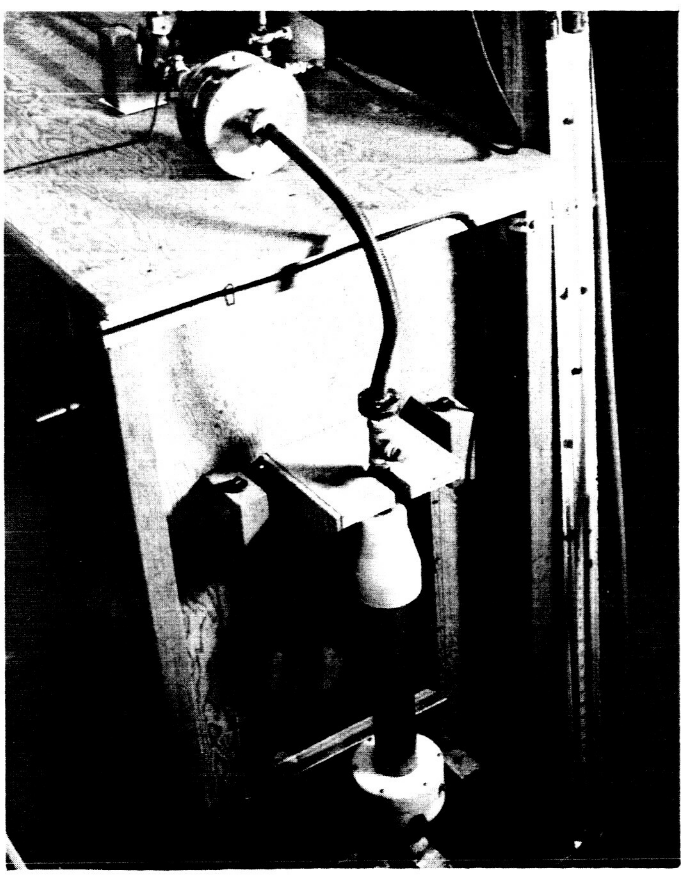


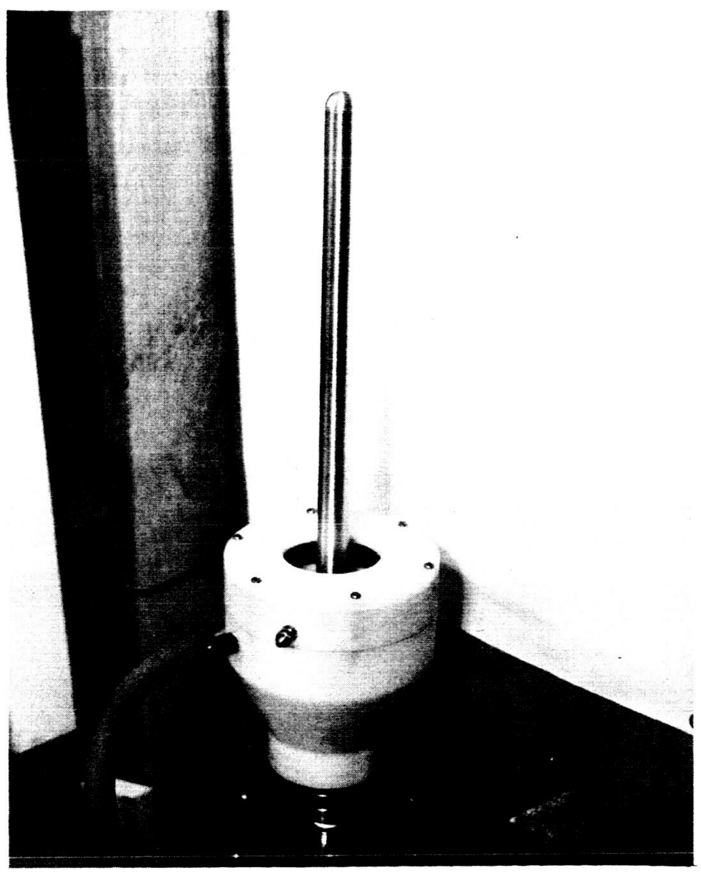
Electrostatic precipitator experimental set-up.



Electrostatic precipitator and downstream filter.



Electrostatic precipitator and upstream filter.



Electrostatic precipitator, discharge wire and high tension electrode.